

Figure 1

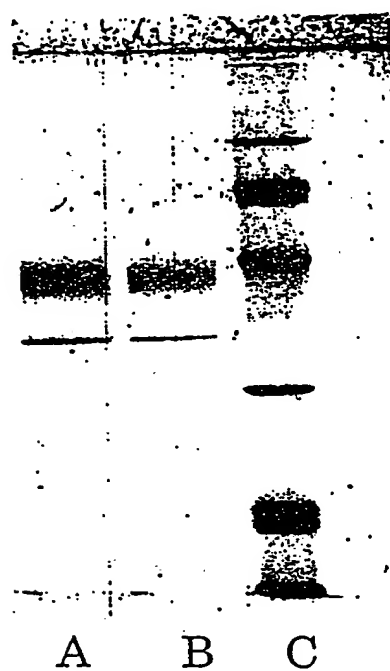


Figure 2

CO I

groups of 10

G.O./peptide

KDIGLLYLVAAGVVG

P.D. 1:MS-AQISDSIEEKGFFTRWFMSTNHDIGVLYLFTAGLAGLISVTLTVYMRMELQHPGVQYMC---LE-
R.S. 1:MADAAIHGHEHRRGFFTRWFMSTNHDIGVLYLFTGGLVGLISVAFVYMRMELMAPGVQFMCAEHLES
B.M. 1:-----M-FINRWLFSTNHDIGTLYLLFGAWAGMVG---TA-LSL-L----IR---AE-L--

P.D. 71:GM-R-LVAD---AAA-E-CTPNAHLWNVVVY-HGILMMFFVVI PALFGGFGNYFMPLHIGAPDMAFPRLN
R.S. 71:GLVKGFFQSLWPSAVENCTPNGLWNVMI-YGHGILMMFFVVI PALFGGFGNYFMPLHIGAPDMAFPRMN
B.M. 71:GQ-PG---TL---LGD---D-QIYNVVV- AHAFVMIFFMVMPIMIGGFGNWLPLMICAPDMAFPRMN

P.D. 141:NLSYWLVCVSLAIASLLSPGGSDQPGAGVGWVLYPPL-ST-TEAGYAMDIAIFAVHVSAGATSILGAIN
R.S. 141:NLSYWLVCVSLAVASLAPGGNGQLSGIGWVLYPPL-ST-SESGYSTDLAIFAVHLSGASSILGAIN
B.M. 141:NMSFWLLPPSFLLLASSMVEAG---AGT---GWTVPPLAGNLAHAGASVDLTIFSLHLAGVSSILGAIN

P.D. 211:IITFTFLNMRAPGMTLFKVPPLFAWVFI TAWMILLSLPVLAGGITMLMDRNFQTFDPAAGGDPVLYQH
R.S. 211:MITFTFLNMRAPGMTLHKVPLFAWSIFVTAWLILLALPVLGAITMLLTDRNFQTFDPAAGGDPVLYQH
B.M. 211:FITTIINMKPPAMSQYQTPLFVWSVMITAVLLLSLPVLAAGITMLLTDRNLNTFFDPAAGGDPILYQH

G.O./PCR

WFFGHPEVYIIILPGFGIISHVSTFS-KKPVFGYLPVYAMLAIGVLGFVVAHMM

>>>>>

<<<<<

P.D. 281:ILWFFGHPEVYMLILPGFGIISHVISTF-ARKPIFGYLPVYAMLAIAFLGFIVWAHMYTAGMSLTQQT
R.S. 281:ILWFFGHPEVYIIVLPAFGIVSHVIATF-AKKPIFGYLPVYAMVAIGVLGFVVAHMYTAGLSLTQQS
B.M. 281:LFWFFGHPEVYIILPGFGMISHIVTYYSCKKEPFGYMGVWAMMSIGFLGFIVWAHMYTVGMDVDTRA

P.D. 351:YFQMATMTIAVPTGIKVFWSIATMGGSSIEFKTPMLWALA--FLFTVGGVTGVVIAQGSILDRVYHDTYYI
R.S. 351:YFMATMTIAVPTGIKIFSWIATMGGSSIELKTPMLWALGFLFLFTVGGVTGIVLSQASVDRIYHDTYYV
B.M. 351:YFTSATMTIAIPTGVKVFWSLATLHGGNIKWSPAMWALGFI FLFTVGGLTGIVLANSILDIVLHDTYYV

P.D. 421:VAHFHYVMSLGAIFAIFAGTYYSIGKMSGRQYPE-WAGQLHFWMFIFGNSLIFFPQHFLGRQGMPPRYID
R.S. 421:VAHFHYVMSLGAIFAGTSGIGKMSGRQYPE-WAGKLHFWMFIFGANLTFPQHFLGRQGMPPRYID
B.M. 421:VAHFHYVLSMGAVFAIMGGFVHWPLFSGYTLNDTWA-KIHFAIMFVGVMNTFFPQHFLGLSGMPPRYSD

P.D. 491:YPVEFSYWMNISSIGAYISFASFLFFIGIVFYTLFAGKPVNPNYWNHADTLEWTLSPPPPEHTFETLP
R.S. 491:YPEAFATWNVVSSLGAFLSFASFLFLGVIFYSL-SGARVTANNYWNHADTLEWTLSPPPPEHTFEQLP
B.M. 491:YPDATWNTISSMGSFISLTAVMLMV-FIIWEAFASKR-EVLTV-DLTTTNLENLNGCPPPYHTFEE-P

P.D. 561:KPEDWDRAQAHR
R.S. 561:KREDWERAPAH-
B.M. 561:TYVN-LK-----

Abbreviations: G.O. *G. oxydans* DSM4025; P.D. *Paracoccus denitrificans*;
R.S. *Rhodobacter sphaeroides*; B.M. Bovine (Mitochondria).
>>>>> <<<<< : amino acid sequences for PCR primers (Fig. 6)
G.O./peptide: amino acid sequence from the purified enzyme
G.O./PCR: amino acid sequence deduced from DNA amplified by PCR (PCR/DNA)

Figure 3

COII

P.D. 1:MAIATKRRGVAAMSLGVATMTAVPALAQDVLGDLFVIGKPVNGGMNFQPASSPLAHDQQWLDHFVLYII
R.S. 1:MRHSTTLTPCATGAA-GLLAATAAAA-QQOTL-E--IIGRPQPGGTGFHGSASPVATQIHWLDGFILVII
B.M. 1:-----MAYPM-Q--LG-FQDATSPIM--EELLHFHD-HT---L-MIVF-L--I

G.O./peptide KASQETHNTFLEIVWTIVFV
G.O./PCR QFTHNTFLEIVWTIVFVVLVFIGAFSLFVLFRQQEFPE-GDI
>>>>>

P.D. 71:TAVTIFVCLLLICIVRNRANPVPARFTHNTFIEVIWTLVPVLILVAIGAFSLPILFRSQEMPNDPDL
R.S. 71:GAITIFVTLILYAVWRFHKRNKVPARFTHNSPLEIAWTIVPILVAIGAFSLPVLFNQQEIP-EADE
B.M. 71:SSLVLYIISLIL--TTKLTHSTNDP----QE--VETIWTILPAIILILIALPSLRILYMMDEI-NNPSL

G.O./PCR VINVEGRSWYWGXE
<<<<<<

P.D. 141:VIKAIGHQWYWSYEYPNDAFDALML-----E-K-----E-ALADAGYSEDEYLLATDNFVVVPVG
R.S. 141:TVKVTGYQWYWGYYEPDEEISFESYMGSPATGGDNRM-SPEVEQQLEAGYTRDEFLLATDTAMVVPVN
B.M. 141:TVKTMGHQWYWSYEY----TDY-EDL--S--L-D-SYM-IPTSE--L-KPG---ELRLLEVDNRVVLPM

P.D. 211:KKVLVQVTATDVIHAWTIPAFVAVQDAVFGRLAQLWFSVDQEGVYFGQCSELGGINHAYMPIVVKAVSQE
R.S. 211:KTVVVQVTGADVHSWTVP-FGVRQDAVFGRLAQLWFAEREGIFFGQCSELGISHAYMPIVVKVSEE
B.M. 211:MTIRMLVSSGDVLSWAVPSLGLKTDAPGRINQTTLMSSRPGLYYGQCSEICGSNHSFMPIVLELVPLK

P.D. 281:KYEAWLAGAKEEFAADASDYLPAFVKLASAE
R.S. 281:AYAAWLEQARGG-TYELSSVLPATPAGV-SVE
B.M. 281:YFEKW-SASML-----

Abbreviations: G.O. *G. oxydans* DSM4025; P.D. *Paracoccus denitrificans*;
R.S. *Rhodobacter sphaeroides*; B.M. Bovine (Mitochondria).
>>>>> <<<<<< : amino acid sequences for PCR primers (Fig. 6)
G.O./peptide: amino acid sequence from the purified enzyme
G.O./PCR: amino acid sequence deduced from DNA amplified by PCR (PCR/DNA)

Figure 4

CO III

P.D. 1:MAHVKNHDYQILPPSIWPFPGAIGAFVMLTGAVAWKGITFFGLPVEGPMFLIGLVGVLYVMFGWADV
R.S. 1:MAHAKNDYHILPPSIWPFMASVGAFVMLNGAVLWMH-----G-S--GPWMGLIGLVVLYTMFGWSDV
B.M. 1:MTH-QTHAYHMVNPSPWPLTGALSALLMTSGLTMW---FHFNMTL----L-MIGLTTNNLTMYQWWRDV

P.D. 71:VNE-GETGEHTFVVRIGLQYGFILFIMSEVMFFVANFWAFIKNALYPMGPDSPIKDGVMPPEGITVTFDPW
R.S. 71:VTE-SLEGDHTFVVRIGLQYGFILFIMSEVIFFSANFWSFFKHALYPMGPESPIIDGIFPPEGIITFDPW
B.M. 71:IRESTFQGHHTPAVQKGLRYGMILFIISEVLFFTGFFWAFYHSSL---AP-TPELGCCWPPTGIHPLNPL

G.O./PCR TWAHHA-IVHGDRKRTAIGLALAIGLGWIFTLCQAYEYIEIVHTE:xxxxxxx
>>>>>

P.D. 141:HLPLINTLILLSGVAVTWAHHAFLVLEGDRKTTINGLIVAVILGVCFTGLQAYEYSHAAGLADTVYAG
R.S. 141:HLPLINTLILLSCGAATWAHHAFLVHENNRDVAWGLALALALGALFTVFQAYEYSHAAGFAGTIYGA
B.M. 141:EVPLLNTSVLLASGVSTWAHHS-LMEGDRKMTQALFITITLGVYFTLLQASEYEAFTISDGVYGS

G.O./PCR xxxxxxxxxxxxxxxD-SIFLLVCLIRILRGAMSAQHVGFEAAWYWHFV

P.D. 211:AFYMATGFHGAHVIIIGTIFLFLVCLIRLLKGAMTQKHVGFEAAWYWHFVDVWVWLFVVIYIWR
R.S. 211:NFFMATGFHGFHVIVGTIFLLVCLIRVQRGHFTPEKHVGFEAAWYWHFVDVWVWLFVVIYIWR
B.M. 211:TFFVATGFHGLHVIIGSTFLIVCFRQLKFHFTSNHFGFEAGAWYWHFVDVWVWLFVVIYIWR

Abbreviations: G.O. *G. oxydans* DSM4025; P.D. *Paracoccus denitrificans*;
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>>>>> <<<<<< : amino acid sequences for PCR primers (Fig. 6)

G.O./PCR: amino acid sequence deduced from DNA amplified by PCR (PCR/DNA)

Figure 5

CO I (target size: approx. 180 bp DNA)

A: W F F G H P → *

5'-TGGTTCTTCGGNCACCC-3'

T T T

B: ← V W A H H M *

3'-CANACCCGNGTAGTATAC-5'

G G

CO II (target size: approx. 180 bp DNA)

A: Q F T H N T → **

5'-CAATTTACNCATAATAC-3'

G C C C

B: ← W Y W G Y E Y *

3'-ACCATAACCCNATACTTAT-5'

G G C

CO III (target size: approx. 300 bp DNA)

A: T W A H H A → *

5'-CANTGGGCNCATCATGC-3'

C C

B: ← W Y W H F V D *

3'-ACCATAACCGTAAAACANCT-5'

G G G

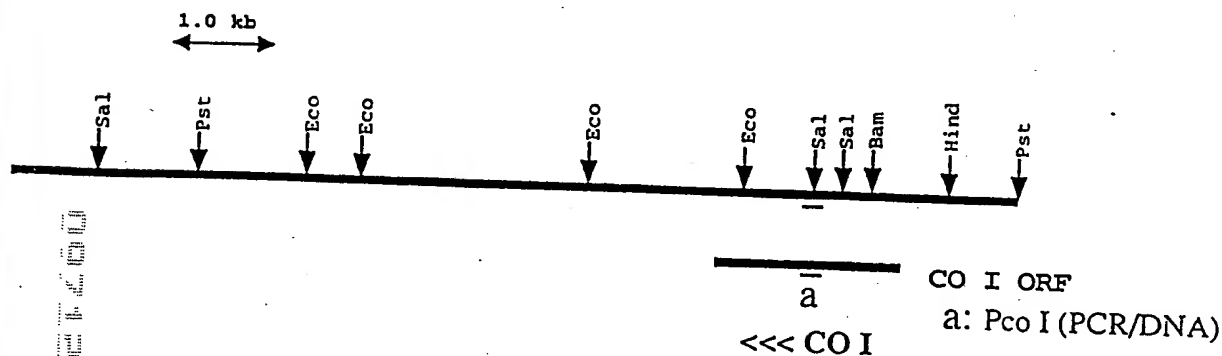
N : A, T, G, C

* : based on consensus sequence

** : based on the peptide sequence

Figure 6

8.0 kb *Pst*I fragment containing CO I ORF of pUCO01



9.3 kb *Eco*RI fragment containing CO II and III ORFs of pUCO23

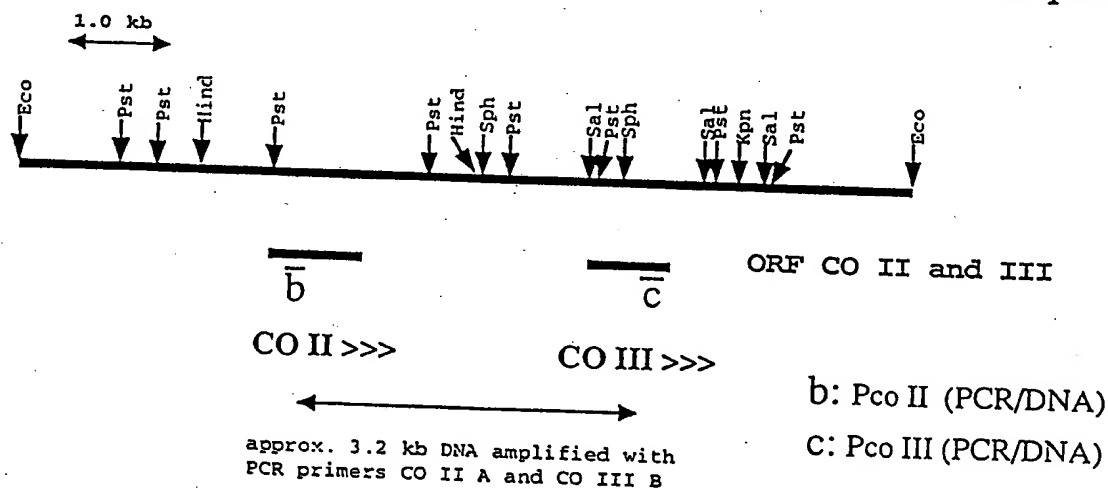


Figure 7

U001333-69221200

B.M. : -----M-FINRWLFSTNHKDIGTLYLLFGAWAGMVGTALESLLIRAELEGPG--TL----L-----G--D-
P.D. : MS-AQI-SDSIEEKGFFTRWFMSTNHKDIGVLYLFTAGLAGLISVTLTVYMRMELQHPGVQYMC---LE-GM-R-LV-A
R.S. : MADAAIHGHE-HDRRGFFTRWFMSTNHKDIGVLYLFTGGLVGLISVAFVYMRMELMAGVQFMCAEHLESGLVKGFQFS
G.O. : MADAAIHGHDHHEKQGFTRWFMSTNHKDIGLLYLVAAGVVGFI SVLFTVYMRLELMDPGVQYMC---LE-G-AR--L--

B.M. : -----D-QIYNVVVT-AHAFVMIFFMVMPIMIGGFGNWLVLPMIGAPDMAFPRMNMNSFWLLPPSFLLLASSM
P.D. : ---DAAAECTPNAHLWNVVVY-HGILMMFFVVI PALFGGFGNYFPLHIGAPDMAFPRMNMNSFWLLPPSFLLLASSM
R.S. : LWPSAVENCTPNGLHWNVMI-YHGILMMFFVVI PALFGGFGNYFPLHIGAPDMAFPRMNMNSFWLLPPSFLLLASSM
G.O. : I-ADASQTCTANGHLWNVMI-YHGILMMFFVGI PALFGGFGNYLMLQIGAPDMAFPRMNMNSFWLLPPSFLLLASSM

B.M. : VEAG---AGT---GWTVPPLAGNLAHAGASVDLTFSLHLGAVSSILGAINFITTIINMKPPAMSQYQTPLFVWSVMITA
P.D. : SPGGSDQPGAGVGVLYPPL-ST-TEAGYAMDLAIFAVHVSIGATSLGAINFITTIINMKPPAMSQYQTPLFVWSVMITA
R.S. : APGGNGQLGSGIGVLYPPL-ST-SESGYSTDLAIFAVHLSGASSILGAINFITTIINMKPPAMSQYQTPLFVWSVMITA
G.O. : APGGDQQLGSGGVLYPPL-ST-REAGYSMDLAIFAVHLSGASSILGAINFITTIINMKPPAMSQYQTPLFVWSVMITA

B.M. : VLLLLSLPVLAAITMLLTDRNLTFFDPAGGGDPILYQHLEWFFGHPEVYIILPGFGMISHIVTYYSKKKEPFGYMG
P.D. : WMILLSLPVLAAITMLLTDRNLTFFDPAGGGDPILYQHLEWFFGHPEVYIILPGFGIISHVISTF-ARKPIFGYLP
R.S. : WLILLALPVLAAITMLLTDRNLTFFDPAGGGDPILYQHLEWFFGHPEVYIILPGFGIISHVISTF-ARKPIFGYLP
G.O. : WLILLALPVLAAITMLLTDRNLTFFDPAGGGDPILYQHLEWFFGHPEVYIILPGFGIISHVISTF-SKKPVFGYLP

B.M. : MVWAMMSIGFLGFIVWAHMYTVGMVDVTRAYFTSATMIIAIPGKVFVSWLATLHGGNIKWSPAMWALGFIFLFTVGG
P.D. : MVLAMAAIAFLGFIVWAHMYTAGMSLTQOTYFQMATMTIAVPTGIKVFVSWLATLHGGNIKWSPAMWALGFIFLFTVGG
R.S. : MVYAMVAIGLVGVVWAHMYTAGMSLTQOTYFQMATMTIAVPTGIKVFVSWLATLHGGNIKWSPAMWALGFIFLFTVGG
G.O. : MVYAMVAIGLVGVVWAHMYTVGMSLTQOTYFQMATMTIAVPTGIKVFVSWLATLHGGNIKWSPAMWALGFIFLFTVGG

B.M. : LTGIVLANSSLDIVLHDTYVVAHFHYVLSMGAFAIMGGFVHWFPLFSGYTLNDTWA-KIHFAIMFVGVNMFTFPQHFL
P.D. : VTGVVIAQGLSDRVYHDTYVVAHFHYVLSMGAFAIMGGFVHWFPLFSGYTLNDTWA-KIHFAIMFVGVNMFTFPQHFL
R.S. : VTGIVLSQASVDYHDTYVVAHFHYVLSMGAFAIMGGFVHWFPLFSGYTLNDTWA-KIHFAIMFVGVNMFTFPQHFL
G.O. : VTGIVLAQGLDRAYHDTYVVAHFHYVLSMGAFAIMGGFVHWFPLFSGYTLNDTWA-KIHFAIMFVGVNMFTFPQHFL

B.M. : GLSGMPRRYSDYDAYTMWNTISSMGSFISLTAVM-L--MVFIWEAFASKREVLTV-DLTTNLEWLNGCPPPVTFTFEE
P.D. : GRQGMPPRYIDYVPEFSYWNMISSIGAYISFASFLFFIGIVF-YTLFAGKPVNVPNYWNEHADTLEWTLSPSPPEHTFET
R.S. : GRQGMPPRYIDYVPEAFATWNVSSLSGAFLSFASFLFFIGIVF-YSL-SGARVTANNYWNEHADTLEWTLSPSPPEHTFET
G.O. : GRQGMPPRYIDYVPEAFALWNKVSYSYGAFLAFASFLFFI-VIFVYTLVAGRRETRPNPWGEFADTLEWTLSPSPPEHTFET

B.M. : -PTYVN-LK-----
P.D. : LPKPEDWDRAQHR
R.S. : LPKREDWERAPAH-
G.O. : LPKRSWDKHPSH-

Homology among amino acid sequences of the CO I subunits of
aa3-type cytochrome c oxidase

	B.M.	P.D.	R.S.
P.D.	52.2	-	-
R.S.	50.6	76.7	-
G.O.	53.3	76.0	78.7

[% homology]

Abbreviations: G.O. *G. oxydans* DSM4025; P.D. *Paracoccus denitrificans*;
R.S. *Rhodobacter sphaeroides*; B.M. Bovine (Mitochondria)

Figure 8

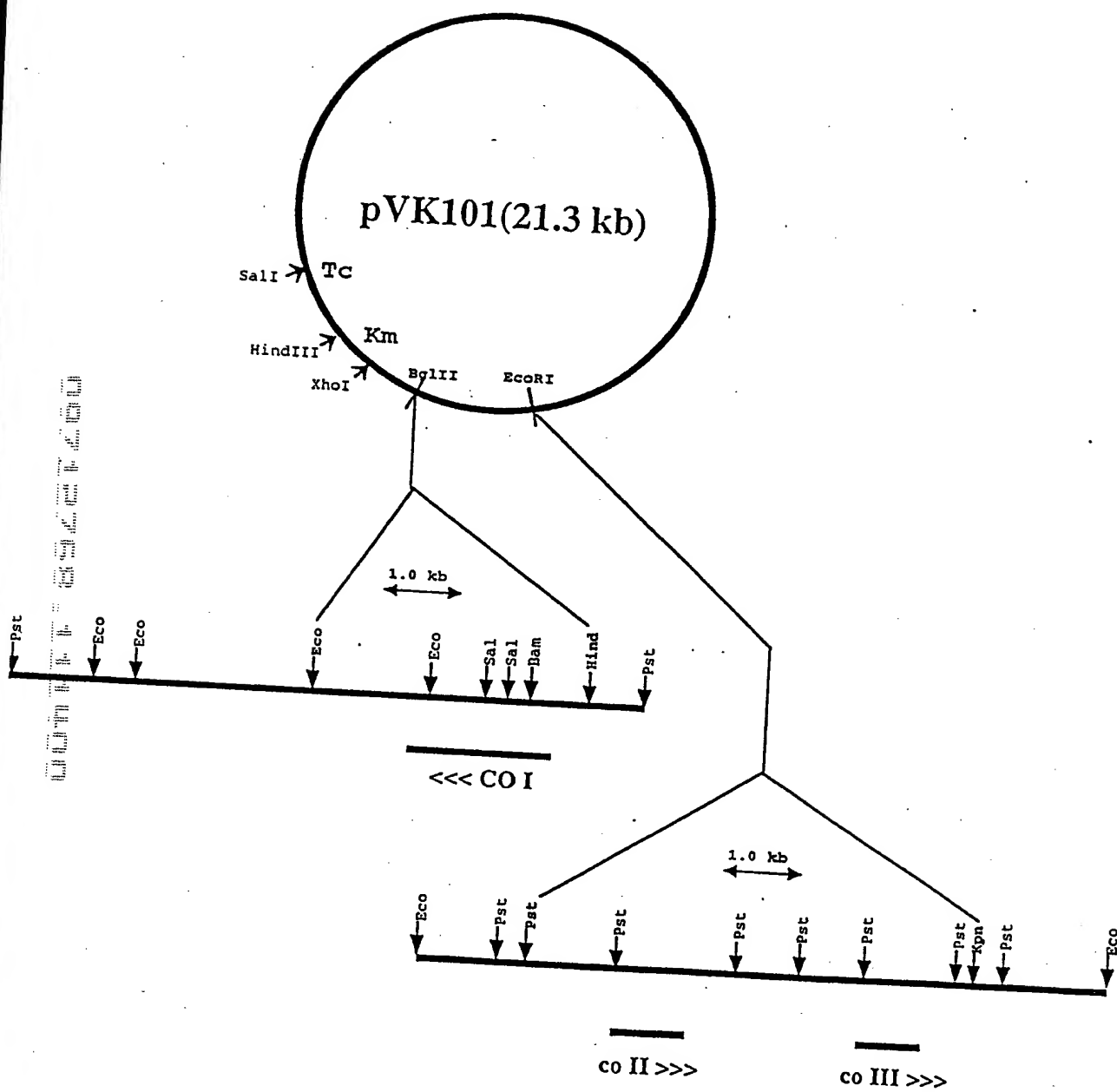


Figure 9